APTAMERS THAT BIND TO NATURAL AND SYNTHETIC CANNABINOIDS

GOVERNMENT SUPPORT

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SEQUENCE LISTING

The Sequence Listing for this application is labeled "SeqList-03Jun20-ST25.txt," which was created on Jun. 3, 2020, and is 8 KB. The Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Bioreceptors are biological macromolecules that are defined by their ability to bind specific ligands. Many bioreceptors have been developed for molecular sensing in a variety of analytical contexts and for therapeutic purposes. Antibodies are the most widely used class of bioreceptors for sensing purposes. Antibodies are typically used both in the scientific community and commercially. Nevertheless, the shortcomings associated with antibodies have fueled interest in alternative reagents.

Aptamers are single-stranded oligonucleotides or peptides that are isolated from randomized nucleic-acid or peptide libraries through an in vitro method termed systematic evolution of ligands by exponential enrichment (SELEX). They have several characteristics that make them favorable as bioreceptors including high chemical stability, ease and affordability of synthesis, and low batch-to-batch variability alongside having high target-binding affinities and well-defined specificity. They have recently gained wide appeal as bioreceptors for biosensing, imaging, and therapeutics due to their low cost of production, ease of modification, chemical stability, and long shelf life. Nevertheless, the isolation of high-affinity oligonucleotide aptamers binding to small molecules is challenging especially when the target is hydrophobic and has limited functional groups for interaction with nucleic acids.

Extensive research has been performed on aptamers regarding their application in remedying a variety of problems in various areas such as medical diagnostics, environmental monitoring, drug detection, and food safety. In tandem, and arguably to a lesser extent, these applied research thrusts are supplemented by fundamental studies of aptamers, which primarily focus on the process by which they are generated (via SELEX) and the exact nature of the interaction of aptamers with their target ligands.

One fundamental and controversial question which has yet to be addressed is: Are there any ligands for which aptamers cannot be isolated? This boundary is well-defined for antibodies; limitations are related to the size of the target as well as its structure and physicochemical properties. There are certain molecules that lack immunogenicity, such as aliphatic or highly hydrophilic compounds such as glucose. Additionally, the antibody generation process is an in vivo process that disallows any precise control over the affinity and specificity of the resulting antibodies. It has been proffered that the in vitro nature of aptamer generation permits the development of bioreceptors for ligands that antibodies cannot be made for with precise control over affinity and specificity. In fact, aptamers have been isolated against targets as small as ions to as large as whole cells, achievements that are unreported for antibodies.

Nevertheless, several accounts suggest that there are certain molecules, to which it is challenging for aptamers to bind. It has been reported that the success rate of SELEX experiments with native DNA libraries is no greater than 30%. Several attempts were made to incorporate new chemistries into nucleic acid libraries to develop aptamers with augmented binding characteristics. Initial success was made by isolating aptamers from libraries containing a single modified nucleotide. Later works developed novel systems that enabled the incorporation of multiple non-canonical moieties in nucleic acid libraries, such as LOOPER and Aegis, to adopt a diverse array of amino-acid-like residues in both DNA and RNA. Although the benefit of using modified libraries in aptamer isolation have been demonstrated in numerous works, SELEX with modified libraries requires additional steps, specifically-engineered polymerases to incorporate modified bases, and/or specialized sequencing methods to identify the sequence of aptamers, all of which increases the cost and reduces the efficiency of SELEX. Meanwhile, aptamers containing custom modified bases may not be commercially accessible. In addition, studies with modified libraries are only limited to proteinaceous targets, and there are no such insights into the limitations that aptamers have with respect to small molecules.

Small-molecule targets are thought to be more difficult to isolate aptamers for, given that they have fewer sites for interaction and binding than macromolecules. It is generally believed, for example, that it is difficult to obtain aptamers for hydrophobic (e.g., cannabinoids such as tetrahydrocannabinol (THC)) and anionic compounds. Attempts have been made using modified libraries with artificial bases; however, these methods are laborious, expensive, and require specially-engineered biomaterials.

Thus, there is a need to develop methods to isolate aptamers that can bind challenging small molecules, e.g., hydrophobic and anionic compounds, and use such aptamers for rapidly and selectively detecting these small molecules.

BRIEF SUMMARY OF THE INVENTION

The subject invention provides methods, assays, and materials for rapid and specific detection of small molecules in a sample, in particular, in both clinical and field settings. In one embodiment, the method for detecting a small-molecule target in a sample comprises contacting the sample with an aptamer-based sensor selective for the small-molecule target, and detecting the small-molecule target in the sample. Advantageously, the aptamer-based sensor comprises one or more aptamers having low nanomolar affinity for their targets and minimal response to structurally-similar compounds.

In one embodiment, the detection of the small-molecule target comprises measuring a signal generated upon assembly of the aptamer-target complex. In another embodiment, the method further comprises determining the concentration of the small-molecule target in the sample.

In one embodiment, the subject invention provides methods for isolating aptamers that specifically bind to small molecules that are challenging for nucleic acids to bind. The success in generating aptamers, according to the subject invention, for these challenging small molecules shows the great impact that the selection conditions can have on the outcome of SELEX experiments. Such results greatly improve confidence in the capability of natural nucleic acid